UNIVERSITY of WASHINGTON
SCHOOL OF MEDICINE
DEPARTMENT OF MICROBIOLOGY
SEATTLE 5

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Dear Dr. Lederberg:

It amazed me to check back on your last letter and find that the date on it was February, 1953. It is possible to report a certain amount of progress with the C. diphtheriae system. I have just finished a manuscript of some of the work and I feel that the best way I can convey the information is to send you a copy. Unfortunately I can't vouch for the speed of the secretarial service so I'll convey the basic conclusions in this letter and forward the paper as soon as possible. I would certainly appreciate your comments.

In essence the hypothesis that lysogenization and toxigenicity conversion, in the particular system under study, are intimately connected seems to have held up. In agreement with your original suspicion the evidence I have accumulated does not conform to transduction. Although Barksdale and Pappenheimer have indicated a similar conclusion I felt a certain mental reservation since in their work and my own the non-toxigenic strain used to propagate the phage was itself susceptible to toxigenicity conversion. Ideally a strain capable of being lysogenized, without being converted to toxigenicity, should have been used, but at present there is no strain which fits this description. Nevertheless it has been possible to show that in a series of 5 consecutive single plaque transfers on the C4 strain that every plaque forming particle, throughout the whole transfer series, still retains the potentiality of conversion to toxigenicity. While the argument is somewhat lengthy it is possible to reason that the dilution effect on a transducing particle would have precluded such results.

The second significant point is that it has been possible to isolate a non-lysogenic revertant from a culture of C4(β). Strain C4(β) is toxigenic and lysogenic; in the reverted strain loss of lysogenicity is accompanied by a loss of toxigenicity. Furthermore these reverted cells when grown and re-exposed to the converting β phage yield toxigenic-lysogenic cells once more. This concommitant gain and loss of toxigenicity and lysogenicity strongly supports the hypothesis entertained above. Reversion has been accomplished in three distinct, widely separated experiments. In one experiment 4 such revertants were obtained and in two others there was 1 per experiment. The screening technique is laborious accounting in part for the low numbers recovered. The technique of obtaining these reverted cells involves exposing the C4(β) cells to a virulent phage mutant and screening the resistant clones for non-toxigenic and/or non-lysogenic cells. As yet we have not isolated a cell which has lost either characteristic independently, although I do not consider this situation impossible subsequent to the initial conversion process.

The third point and one which has opened up the problem is the isolation of a phage which lysogenized the C4 cells but does not convert them to toxigenicity. We are now exploring the ramifications of this observation. It is obvious at once that conversion to toxigenicity is a phage specific phenomenon, that lysogenization with phage does not necessarily mean toxigenicity conversion. The other question as to whether toxigenicity is always related to lysogenization with a particular type or types of phage is really not answered yet if one expands this phenomenon to include all toxigenic C. diphtheriae. The difficulties which can arise in demonstrating the presence of a prophage in a given cell may

make an answer to this question well nigh impossible to obtain. For example "mutation" at a prophage locus, assuming now that the locus is chromosomal, could result in the retention of toxigenicity but loss of inductive properties on the part of the prophage. Or it is possible that a non-toxigenic C. diphtheriae cell may mutate to a condition of toxin production independent of phage action. To my mind a method of discriminating between these two mechanisms with the same demonstrable end result is not yet at hand.

Beyond the point of having isolated this new phage there is some interesting data accumulating but it is by no means at the quotable stage. We have found that a cell lysogenized by the new phage (lysogenic but non-toxigenic) is still susceptible to action by the β converting phage. At present we are involved in attempting to isolate the doubly lysogenic cells and any possible variants therefrom. It does seem certain that a cell carrying the new phage can be converted to toxigenicity by exposure to β. We have not critically determined the serological relationship of these two phages, but using a β antiserum which had a rather low neutralizing titer it appeared that the rate of neutralization of these two phages was identical. We are in the process of trying to get better antisera, but low titers and rapid inactivation of the diphtheria phages makes this somewhat difficult. One other interesting point which we are striving to nail down is whether conversion to toxigenicity is a function of the entire prophage or whether it is a function of a subunit. At the moment there are some indications that conversion to toxigenicity is a sub-unit function and I hope we will have more information on the point very soon.

Having first spewed forth what I know, due credit goes to Barksdale for the simultaneous isolation of a phage which lysogenized but which does not convert to toxigenicity. Furthermore he has written about some interference effects of another phage on the ability of β to convert to toxigenicity when cells are doubly lysogenized. Of course he should speak for himself, but I simply wanted to indicate that there is considerable ferment at the moment and I would suggest that you write directly to him for the information. Our correspondence has been regular and to me very rewarding. His present address is, Institut Pasteur, 28 Rue du Dr. Roux, Paris, France c/o Service Lwoff. There are a few other workers who have corresponded with me, but of these the only suggestion that progress was being made came from Dr. L. F. Hewitt. He did not relay any specific information. A note from a Dr. Hatano in Japan suggests that he, too, has observed reversion from the toxigenic-lysogenic state to one of non-toxigenicity and non-lysogenicity.

I shall try to get the manuscript to you soon and would be grateful for any comments and suggestions you might have in pursuit of the work.

Sincerely yours,

Neal B. Groman.

Neal B. Groman
Assistant Professor